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Green Synthesis of Phloroglucinol: Exploiting Pseudomonas fluorescens and Scale-Up

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14. ABSTRACT

Through a combination of metabolic engineering and reaction engineering, a new generation of phloroglucinol synthesizing microbe has been developed and evaluated using resin-based extractive fermentation process. Phloroglucinol synthase, phlD, from P. fluorescens PF-5 had been cloned and expressed in E. Coli PG1/pBC2.274. The isolated genes product has been unambiguously identified as phloroglucinol synthase using electrospray ionization tandem mass spectroscopy. The expression of phlD in E. Coli PG1/pBC2.274 under fermentor-control conditions generated inclusion bodies of inactive protein. By attenuating the expression level of phlD in E. coli, a more stable phloroglucinol production E. coli strain, PG1/pKIT10.080, has been developed and capable of producing 25 g/L of phloroglucinol under fermentor-controlled resin-based extractive cultivation conditions. The use of resin-based extractive technique facilitates the removal of toxic phloroglucinol product from the fermentation process as it is being formed allowing for maximum production of phloroglucinol in the E. coli host. In contrast, microbial synthesis of phloroglucinol by a wild-type P. fluorescens species under similar culturing condition produced only 0.1g/L phloroglucinol.

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Abstract/Project Summary: Through a combination of metabolic engineering and reaction engineering, a new generation of phloroglucinol synthesizing microbe has been developed and evaluated using resin-based extractive fermentation process. Phloroglucinol synthase, *phlD*, from *P. fluorescens* PF-5 had been cloned and expressed in *E. Coli* PG1/pBC2.274. The isolated gene's product has been unambiguously identified as phloroglucinol synthase using electrospray ionization tandem mass spectroscopy. The expression of *phlD* in *E. Coli* PG1/pBC2.274 under fermentor-control conditions generated inclusion bodies of inactive protein. By attenuating the expression level of *phlD* in *E. coli*, a more stable phloroglucinol production *E. coli* strain, PG1/pKIT10.080, has been developed and capable of producing 25 g/L of phloroglucinol under fermentor-controlled resin-based extractive cultivation conditions. The use of resin-based extractive technique facilitates the removal of toxic phloroglucinol product from the fermentation process as it is being formed allowing for maximum production of phloroglucinol in the *E. coli* host. In contrast, microbial synthesis of phloroglucinol by a wild-type *P. fluorescens* species under similar culturing condition produced only 0.1g/L phloroglucinol.

Scientific Technical Objectives: A new generation of phloroglucinol synthesizing microbe has been evaluated using resin-based extractive fermentation at 1 L scale. Strategies to understand *in vivo* phloroglucinol synthase expression in this genetically engineered *E. coli* were examined. Improving phloroglucinol synthase activity is essential to increase the microbial phloroglucinol synthesis titer and yield. Strategies to identify novel phloroglucinol synthases were therefore explored. It is also believed that expression of phloroglucinol synthase *phlD* gene inside its native *Pseudomonas fluorescens* Pf-5 will likely deliver higher expression level and activity of this gene product. Constructing a phloroglucinol synthesizing *P. fluorescens* was therefore pursued.

Approach: Coupling metabolic engineering and reaction engineering, improved phloroglucinol synthesizing *E. coli* PG1/pKIT10.080 was evaluated under resin-based extractive fermentor-controlled conditions. Heterologous expression of *P. fluorescens* Pf-5 *phlD* gene in *E. coli* presents numerous complications. 2D protein gel electrophoresis and tandem mass spectroscopy were used to examine the relatively short catalytic lifetime and low activity of PhlD. In an attempt to identify better phloroglucinol synthases, the genetic diversity of *phlD* was explored. Bioinformatics analysis of potential *phlD* candidates in various organisms with significant amino acid sequence identity to our currently used Pf-5 *phlD* were examined. In parallel with these efforts, microbial synthesis of phloroglucinol in *P. fluorescens* was examined. Defined minimal salt medium was formulated to enable high density cultivation of *P. fluorescens* Pf1.162/pJA2.232 in the fermentor.

Accomplishments: Under optimized resin-based extractive fermentation, *E. coli* PG1/pKIT10.080 synthesized 25 g/L of phloroglucinol. Coupling 2D protein gel electrophoresis and tendem mass spectroscopy, it was determined that heterologously expressing PhlD-encoded phloroglucinol synthase under fermonetor-controlled conditions led to significant formation of inclusion bodies. In attempts to identify phloroglucinol synthase alternatives using bioinformatics approach, five active phloroglucinol synthases were identified. High-cell density cultivation of *P. fluorescens* was achieved in fermentation vessels using minimal salts medium. Phloroglucinol production was observed by culturing *P. fluorescens* Pf1.162/pJA2.232 under defined fermentor-controlled conditions.

Conclusions: The production of pholorogucinol is easily achieved from glucose, and the remaining technical issue is now clear; expression of the phlD enzyme in its properly folded, catalytically active state. Draths is actively pursuing the necessary business issues for access to the Pfenex expression system, which is known to be capable of functional expression of pseudomonad proteins such as phlD.

Significance: Phloroglucinol continues to attract interest at commerical scale and Draths continues to receive inquiries for commercial supply. Production of phloroglucinol from biomass sugars enjoys no viable competition from the petrochemical industry. This commercially useful molecule is expected to be very attractive target for the bio-based chemicals industry.

Publications: None

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